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Use of recombinant human erythropoietin in combination with parenteral iron in the treatment of postpartum anaemia

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Abstract. The authors compared the effect of recombinant human erythropoietin (rhEPO) in combination with iron with that of iron therapy only in the treatment of postpartum anaemia. Ninety patients (30 patients/group) received either rhEPO (300 $U kg^{-1}$, i.v. or s.c., once) and iron (parenteral and oral), or iron therapy only. Erythropoiesis was assessed by haemoglobin and haematocrit increase, absolute reticulocyte counting and reticulocyte flow cytometry. Ferrokinetics was assessed by serum ferritin, transferrin and transferrin saturation measurements. There was no difference before therapy for baseline haematological values or iron status. Patients with endogenous EPO levels below 145 $mU mL^{-1}$ had a significant benefit from intravenous rhEPO administration with highest haematocrit and haemoglobin levels 4 and 14 days after therapy. rhEPO-treated groups showed a higher absolute reticulocyte count 1 and 4 days after therapy and an elevated percentage of high fluorescent reticulocytes (HFRs). Parenteral iron therapy caused a significant increase of ferritin and transferrin saturation, while transferrin concentration decreased. Ferritin and transferrin levels were lowest after i.v. administration of rhEPO, 1 and 4 days after therapy. C-reactive protein concentration was highest in patients who underwent caesarean section until the end of the observation period. A single dose of rhEPO in combination with iron was more effective in treating postpartum anaemia than iron therapy only, in patients who had low EPO levels despite peripartal blood loss. Postpartum low endogenous EPO levels might be a consequence of inhibiting inflammatory cytokines that are released after spontaneous or operative deliveries.

Keywords. Endogenous erythropoietin, erythropoiesis, ferrokinetics, postpartum anaemia, recombinant human erythropoietin, reticulocyte flow cytometry

Introduction

Safe and effective treatment of postpartum anaemia is a frequent problem in obstetrics. Depending on the severity of blood loss anaemic patients may present an increased morbidity, including cardiovascular symptoms, dizziness, fatigue, infections and lactation problems [1–3].

Classic treatment of postpartum anaemia was iron therapy only and/or blood transfusion. Owing to its considerable risks blood transfusion should be restricted whenever possible in young and otherwise healthy patients [4,5]. The effect of oral iron might be reduced as a result of its limited resorption, gastrointestinal side-effects when given in higher dosages and the dependence on the patient's compliance [6–9]. In addition, it is known that iron therapy has little effect on haemoglobin concentration in cases of disturbed cellular iron mobilization as seen within 24 h after major and minor surgery and after inflammatory states with elevated cytokine levels [10]. As erythropoiesis might be blunted shortly after operative or vaginal deliveries owing to inflammatory injuries [11,12], iron therapy alone may often fail to treat postpartum anaemia sufficiently.

Recombinant human erythropoietin (rhEPO), which has been successfully used as substitution therapy in renal anaemia since 1986, is also effective in stimulating erythropoiesis after acute blood loss in patients without renal disease, e.g. during autologous blood donations or in perioperative settings [13–20]. rhEPO is also effective if erythropoiesis is blunted, as a result of inadequately low endogenous EPO levels, e.g. during chronic and acute inflammatory states [10,12,21].

It has been shown that oral iron does not prevent functional iron deficiency during rhEPO therapy. Adequate iron supply, which is achieved mainly by administration of parenteral iron, is a prerequesite for successful stimulation of erythropoiesis with rhEPO [22–24]. We have already reported on several studies using rhEPO in combination with oral iron for the treatment of postpartum anaemia [25,27,36].

In the following study we used rhEPO in combination with both, parenteral and oral iron supplementation, to compare its effectiveness in treating postpartum anaemia with that of iron therapy only.

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Materials and methods

Study protocol and patient selection

This was a prospective, randomized and controlled study with 90 patients. All women gave informed consent, and the project was approved by the hospital ethics committee. Inclusion criteria were postpartum haemoglobin $<10{\cdot}0$ g dL^{-1} 48–72 h after delivery, normal cardiac and renal function, oral iron substitution during pregnancy. Exclusion criteria were pregnancy anaemia, peripartal infection (elevated C-reactive protein level or fever), peripartal blood transfusion, haematological disease, e.g. thalassaemia, previous myelosuppressive medication, history of thromboembolism, haemosiderosis, iron intolerance or rheumatoid polyarthritis.

Patients were randomly assigned using sealed envelopes to one of three groups:

Group I (control) ($n = 30$): saccharated iron (Ferrum Hausmann®, Vifor, St. Gallen, Switzerland), 100 mg (5 mL), once i.v. and oral iron sulphate, 160 mg elemental iron day^{-1} + folic acid, 0·7 mg day^{-1} for 6 weeks.

Group II ($n = 30$): rhEPO (Eprex®, Cilag Schaffhausen, Switzerland), 300 U kg^{-1} b.w. (~2 mL), once s.c. + saccharated iron, 100 mg (5 mL), once i.v., and oral iron sulphate, 160 mg elemental iron day^{-1} + folic acid, 0·7 mg day^{-1} for 6 weeks.

Group III ($n = 30$): rhEPO, 300 U kg^{-1} b.w. once i.v., + saccharated iron, 100 mg (5 mL), once i.v., and oral iron sulphate, 160 mg elemental iron day^{-1} + folic acid, 0·7 mg day^{-1} for 6 weeks.

Treatment was started 48–72 h after delivery. rhEPO and iron were injected through an intravenous catheter, first iron (5 mL) over 10 min, then rhEPO (~2 mL) as a bolus at room temperature. Between iron and rhEPO, saline solution (5 mL) was injected to clean the tube. rhEPO was injected s.c. into the upper third of the thigh in group II. Blood samples (25 mL/sample) were taken before therapy and on days 1, 4, 14 and 42 after start of the therapy. Vital signs were recorded daily until discharge.

Laboratory evaluations

Complete blood count and red cell indices were measured in blood collected in potassium EDTA (Technicon H1® blood analyser system /Miles Diagnostic, Tarrytown, USA). The absolute reticulocyte count was determined by automated flow cytometry (Sysmex R 2000®, TOA Medical Electronics, Watabe, Japan), simultaneously with the percentage of reticulocytes at different stages of maturity, namely high (HFR), middle (MFR) and low (LFR) fluorescent ratio reticulocytes according to their RNA content. HFR reticulocytes are young and immature reticulocytes with a high RNA content that are prematurely released from the bone marrow following stimulus of erythropoiesis, e.g. after rhEPO administration, and that change to mature reticulocytes circulating in the blood [28–31].

Iron status

Serum iron and transferrin concentrations were measured by standard autoanalyser techniques. Transferrin saturation was calculated using the formula: serum iron concentration $\times 100$/ transferrin concentration $\times 1{\cdot}7$.

Serum ferritin was measured by standard fluoroimmunometric assay. Because there was considerable variation in the initial individual values, the results were normalized and expressed as a percentage of the starting value for each patient.

C-reactive protein concentration (CRP) was determined by the high-performance liquid chromatography method. Serum EPO levels were determined by sandwich ELISA as described by Eckardt *et al.* [32].

Statistics

Groups were compared by using a one way analysis of variance. Subsequent differences between groups were evaluated by using unpaired two-tailed *t*-tests, which were corrected for multiple comparisons by using the Bonferroni–Dunn method. Statistical significance was

Table 1. Baseline data before treatment in postpartum patients who received iron (group I = control), rhEPO s.c./iron (group II) and rhEPO i.v./iron (group III)

	Iron	rhEPO s.c.	rhEPO i.v.	*P*	(Normal range)
Blood loss (mL)	566 ± 55	643 ± 58	657 ± 78	NS	
SOT (h)	53·6 ± 16·2	56·5 ± 15·8	53·1 ± 16·4	NS	
EPO level (mU mL)$^{-1}$	122 ± 20	168 ± 25	137 ± 18	NS	(8–22 mU mL)$^{-1}$
Hb prepartum (g dL)$^{-1}$	11·8 ± 0·2	11·6 ± 0·2	11·8 ± 0·2	NS	(10·5–12·0 g dL)$^{-1}$
Hb postpartum (g dL)$^{-1}$	8·8 ± 0·2	8·7 ± 0·2	8·9 ± 0·3	NS	
PCV prepartum (%)	26·7 ± 0·7	26·0 ± 0·8	26·9 ± 0·9	NS	(31·5–36·0%)
PCV postpartum (%)	35·6 ± 0·6	34·9 ± 0·5	35·2 ± 0·6	NS	
Ferritin (μg L)$^{-1}$	30·2 ± 17·1	26·1 ± 19·7	40·3 ± 24·5	NS	(12–136 μg L)$^{-1}$
Transferrin saturation (%)	15·8 ± 12·1	17·1 ± 16·1	16·5 ± 15·5	NS	(20–50%)
CRP (mg L^{-1})	61·7 ± 6·1	69·9 ± 9·7	77·3 ± 10·5	NS	(<10 mg L)$^{-1}$

SOT, start of treatment after delivery; EPO, EPO level before treatment; PCV, packed 123–130 cell volume; CRP, C-reactive protein. Mean ± SEM.

Table 2. Baseline data of patients with low EPO levels and patients with high EPO levels before treatment

	Blood loss (mL)	EPO (mU mL^{-1})	Hb (g dL)$^{-1}$	PCV (%)	CRP (mg L)$^{-1}$	Ferritin (μg L)$^{-1}$
EPO < 145 mU mL^{-1} (n = 36)	547 ± 46 (200–1400)	80 ± 5 (30–131)	8·8 ± 0·1 (6·8–9·9)	26·5 ± 0·4 (20–32)	70 ± 7 (5–166)	35 ± 3 (2–80)
EPO > 145 mU mL^{-1} (n = 54)	802 ± 93 (300–1800)	241 ± 3* (145–490)	7·9 ± 0·2** (5·5–9·3)	23·8 ± 0·6** (16·4–28)	72 ± 10 (8–156)	30 ± 4 (7–88)

Values are given as mean ± SEM and range *P < 0·05, **P < 0·01, *P 0·05 Mann–Whitney test.

accepted at a P value less than 0·05. Data are presented as mean ± SEM.

In an effort to determine whether any pretreatment characteristics of the patients were predictive of response to rhEPO therapy, a comparison of baseline characteristics of the responder population with patients who did not respond to rhEPO was performed. The Mann–Whitney test was used to compare patients with low endogenous EPO levels before treatment with those who had high EPO levels.

RESULTS

Patients' characteristics

Table 1 displays baseline values of erythropoiesis and iron status. No differences were observed concerning iron status, endogenous EPO levels or haemoglobin between the groups. All women had normal prepartum haemoglobin and haematocrit levels. Postpartum haemoglobin ranged from 8·4 g dL^{-1} to 8·7 g dL^{-1} for an average estimated blood loss of 500–700 mL. Mean start of treatment was between 53·5 and 56·5 h after delivery. Owing to the acute blood loss, endogenous EPO levels were elevated before therapy (122–168 mU mL)$^{-1}$. Serum ferritin levels were in the lower normal range (28·1–40·3 μg L)$^{-1}$ for all three groups. A total of 19 (17%) patients had proven iron deficiency (ferritin < 12 μg L)$^{-1}$ and 36 (40%) showed latent iron deficiency (transferrin saturation < 20%) before therapy. CRP concentration was elevated in all groups before therapy.

Predictive factor for the response to rhEPO

Comparing pretreatment characteristics of patients, it was found that low endogenous EPO levels were most predictive for a benefit of rhEPO treatment. The calculated mean cut-off value for this subgroup of patients was below 145 mU mL^{-1}.

Mean endogenous EPO levels were significantly lower in this group (80·0 vs. 241·0 mU mL^{-1}; P < 0·01) before treatment, while there was no difference in pretreatment ferritin levels or CRP levels (Table 2). As shown in Table 2 mean haemoglobin and haematocrit values were lower in the 'high' EPO level group before therapy. However 14/36 patients in the 'low' EPO level group had haemoglobin levels below 8·5 g dL^{-1}. CRP concentration was elevated in both groups before therapy. Ferritin levels were within the normal range for both groups before therapy. The estimated average blood loss was higher in patients with high EPO levels.

Comparing the mode of delivery in the two subgroups it was found, that the 'low' EPO level group had a higher percentage of operative deliveries (63·3% vs. 54·1%; P < 0·05).

Erythropoietic response

Regarding therapy response of all patients, group III (rhEPO i.v.) showed higher haemoglobin and haematocrit levels 14 days after therapy (Fig. 1A & B). No difference was detected between group II (rhEPO s.c.) and controls. If patients were selected according to their pretreatment EPO levels it was seen that patients with endogenous levels below 145 mU mL^{-1} (n = 36) had a significant benefit from i.v. rhEPO administration with significantly higher haematocrit and haemoglobin levels 4 and 14 days after therapy compared with controls and group II. In group III mean haemoglobin concentration 4 days after therapy was 10·3 (± 0·2) g dL^{-1} vs. 9·1 (± 0·2) g dL^{-1} in the control group. In group III 8/11 patients had haemoglobin levels over 10·0 g dL^{-1} 4 days after therapy compared to 3/13 patients in the only iron-treated group. Fourteen days after therapy mean haemoglobin values were 12·0 (± 0·3) g dL^{-1} vs. 11·0 (± 0·1) g dL^{-1} in the control group.

Highest absolute reticulocyte counts were seen 4 days after therapy (Fig. 1C), with the highest increase being found in group II. Group III had significantly higher reticulocyte counts compared with controls on day 1.

The high fluorescent reticulocyte count (HFR) increased until day 4 (Fig. 1D). In the groups receiving rhEPO, HFR count was significantly higher compared with controls on days 1 and 4 after therapy, indicating higher bone marrow activity in these groups. In accordance with the decrease of the total reticulocyte count the HFR count decreased in all groups, indicating the maturation of young reticulocytes into mature cells and normal bone marrow proliferation.

Iron metabolism

The day after parenteral iron therapy, there was a significant increase in ferritin levels (Fig. 2A). This

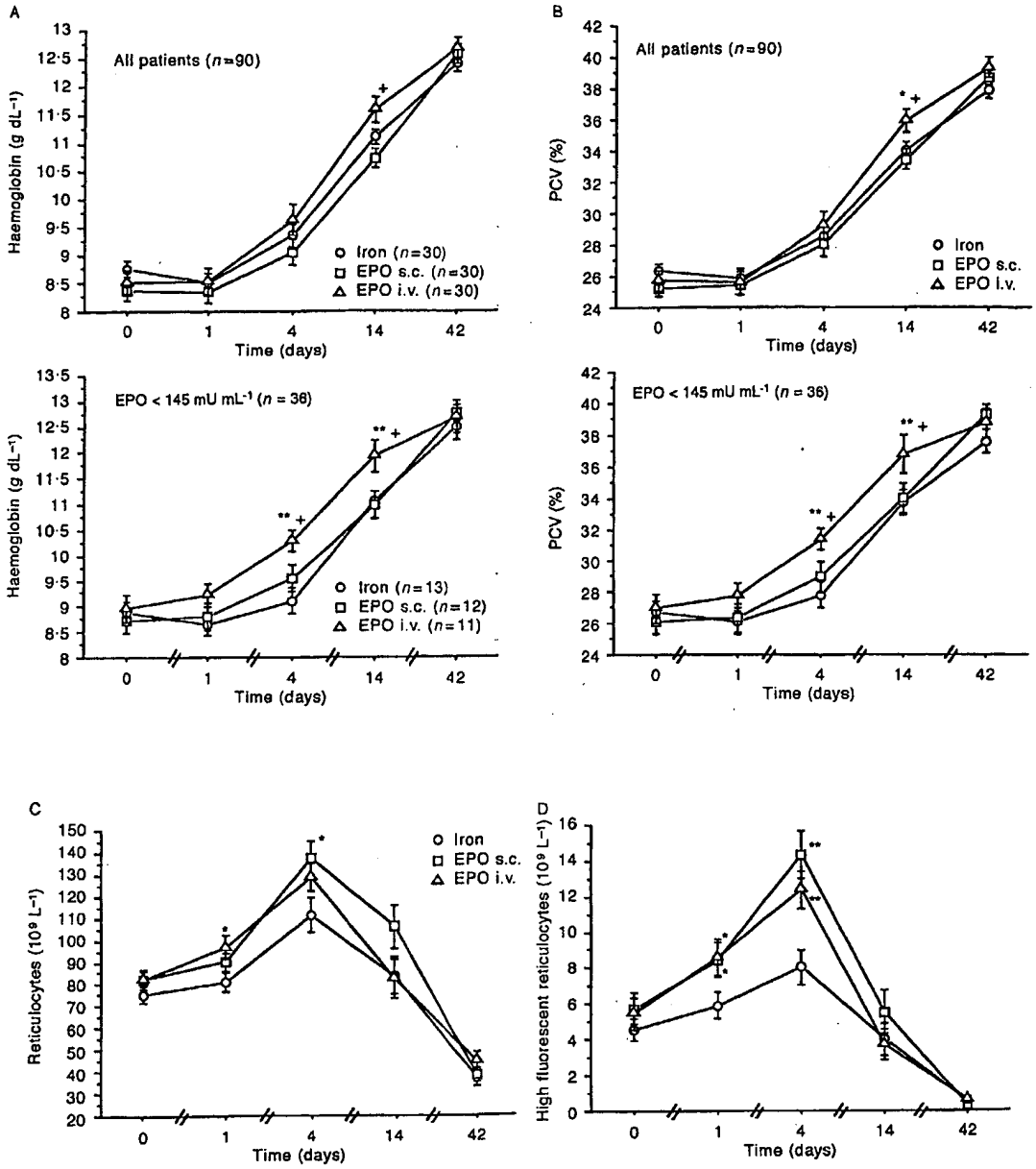

Figure 1. Erythropoietic activity in postpartum patients who received iron (control), rhEPO s.c. and iron (group II) and rhEPO i.v. and iron (group III), expressed as a haemoglobin concentration (A), packed cell volume (B), absolute reticulocyte count (C) and high fluorescent reticulocyte count (D). (A and B). Further subgroups of patients with low EPO levels (< 145 mU mL^{-1}) before treatment. Values are expressed as means ± SEM. $^{**}P < 0.01$ vs. control, $^{*}P < 0.05$ vs. control; + $P < 0.05$ vs. group II.

increase was significantly lower in group III compared with group II and controls. Thereafter groups II and III showed a continuous fall in ferritin levels, whereas group I showed a plateau phase until day 4 . All three groups had lower ferritin levels 6 weeks after treatment compared with pretreatment values.

Transferrin saturation increased rapidly as a result of the increasing serum iron levels and was lowest in group III on day 1. Owing to increased iron mobilization and decreasing serum iron levels, the transferrin saturation fell until day 4 and normalized thereafter (Fig. 2B). After treatment with rhEPO i.v., group III showed a rapid decrease in transferrin levels with significantly lower levels on days 1 and 4 after therapy compared with the control group and group II (Fig. 2C). In the control group transferrin levels showed a plateau phase until day 1. Group III had significantly higher transferrin saturation 6 weeks after therapy compared with the control group. Transferrin levels were within the normal range for all groups at the end of the observation period.

C-reactive protein concentration (CRP)

CRP concentration was elevated during the first week in all groups and was still slightly elevated 14 days after therapy (Fig. 3A). No significant difference in CRP concentration between groups was detected.

There was a remarkable difference in the course of CRP concentration comparing patients who had spontaneous or vaginal operative deliveries and patients who underwent caesarean section (Fig. 3B). Before therapy, these patients had significantly higher CRP concentrations with peak values of 120 (± 10) $mg\,L^{-1}$ compared with 58 (± 9) $mg\,L^{-1}$ for vaginal operative and 55 (± 6) for spontaneous deliveries 48–72 h after delivery and until the end of the observation period. CRP concentration in patients who underwent vaginal operative procedures had slightly higher CRP levels than patients with spontaneous deliveries, but no significant difference was detected.

Platelet count

All groups had normal platelet counts before therapy. The platelet count increased until day 14 in all groups and was highest in the rhEPO-treated groups but within the normal range and not significantly different from the control group.

Clinical evaluation

No blood pressure increases or thromboembolic complications were observed. No anaphylactic reaction to saccharated iron was seen. Two patients mentioned a 'warm' sensation during injection of iron that disappeared quickly and spontaneously after a few minutes. Twenty-seven patients felt a 'metallic' taste during injection that vanished after some minutes. Ten patients complained of a burning sensation during rhEPO injection in the thigh at room temperature. Overall, the therapy was well tolerated, as estimated clinically and by the patient's opinion. After study admittance, none of the patients needed blood transfusion in addition to iron or iron and rhEPO treatment.

Discussion

This study investigated the effectiveness of rhEPO in stimulating erythropoiesis in anaemic patients. As seen by the release of mature and immature reticulocytes, it was shown that a single dose of rhEPO and parenteral iron stimulates erythropoiesis, even if pre-existing EPO levels are high after acute peripartal haemorrhage. A similar effect of exogenous rhEPO has been described in autologous blood donation programmes, in perisurgical settings and for Jehovah's Witnesses refusing blood transfusion. [13,15,17,18–20]. Concerning haemoglobin and haematocrit increase for all treated patients, difference between rhEPO-treated patients and only iron-treated patients was small or even non-existent, if rhEPO was administered subcutaneously.

Searching for pretreatment criteria that would indicate which patients would benefit from rhEPO administration, we found that in the group of patients who had endogenous EPO levels below 145 $mU\,mL^{-1}$, there was a significant difference in haemoglobin and haematocrit increase between rhEPO i.v. and only iron-treated patients. In this subgroup most patients had haemoglobin levels over 10·0 $g\,dL^{-1}$ after 4 days with an average increase of 1·3 $g\,dL^{-1}$, which corresponds to approximately 1–2 blood units. Our result is consistent with the finding that patients with EPO levels that are considered low for the rate of anaemia benefit from exogenous EPO administration [26,34,39]. One known factor for an impaired EPO production despite hypoxic stimulus is an inflammatory state causing a release of cytokines, which inhibit erythropoietin production and erythropoietin action on the bone marrow [10,21,26,35].

Since delivery causes a physiological inflammatory reaction, which is indicated by elevated CRP levels [11,12], and which is aggravated by surgical interventions, many of these patients may present elevated levels of inflammatory cytokines such as interleukin 1 (IL-1) and tumour necrosis factor alpha, which supress erythropoiesis and inhibit EPO production.

As seen in our patients after caesarean sections, CRP levels are considerably higher compared with vaginal deliveries and it is likely that these patients present excessive levels of inhibitory cytokines. Because these patients might have blunted EPO production rather than a lack of iron, iron alone will not stimulate erythropoiesis sufficiently.

In addition, since the release of reticuloendothelial iron is impaired during inflammatory states, exogenous iron is needed [10,37].

For haematocrit levels below 30%, the rate of red blood cell production depends on several sources of iron [38]. By administering intravenous iron, high iron plasma levels are reached and enough iron is supplied

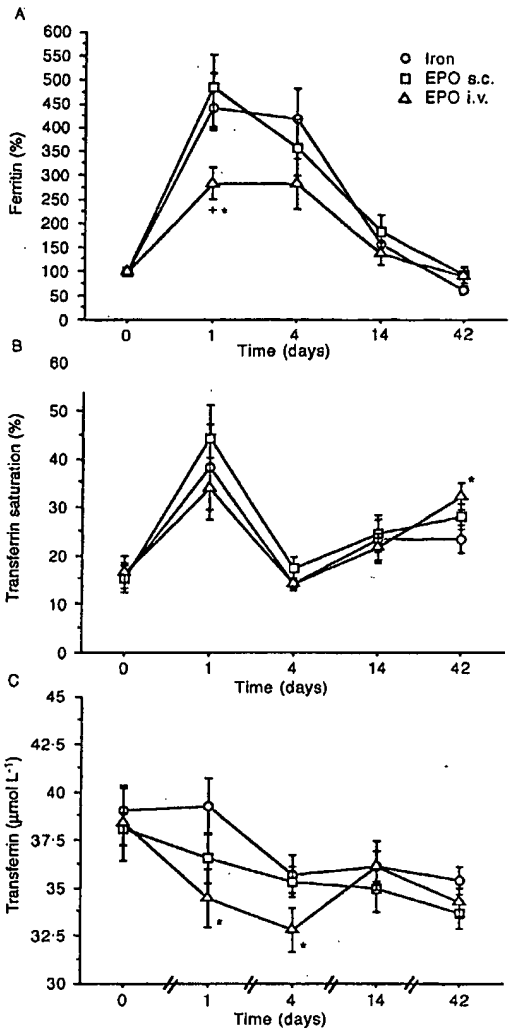

Figure 2. Iron metabolism in postpartum patients who received iron (control), rhEPO s.c. and iron (group II) and rhEPO i.v. and iron (group III), measured by serum ferritin (A) transferrin saturation (B) and transferrin concentration (C). Values are expressed as means ± SEM. $*P < 0.05$ vs. control; $+P < 0.05$ vs. group II. ○, Iron; □, EPO s.c.; △, EPO i.v.

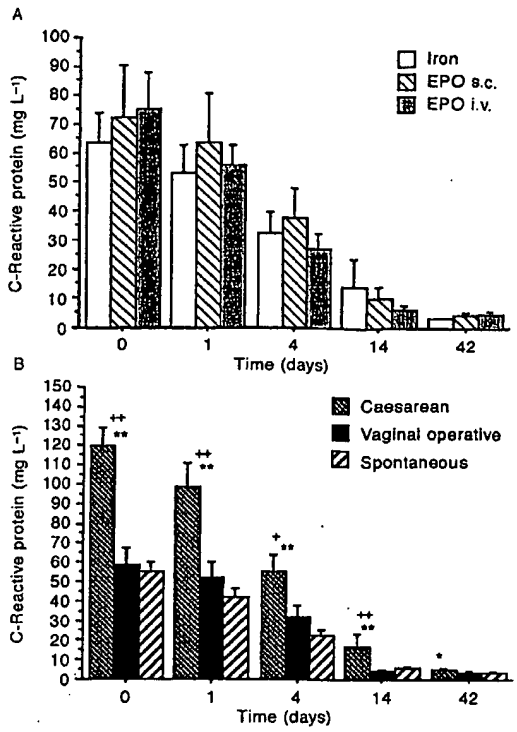

Figure 3. (A) C-reactive protein concentration in postpartum patients who received iron (control), rhEPO s.c. and iron (group II) and rhEPO i.v. and iron (group III). (B) The course of CRP concentration depending on the mode of delivery. Values are expressed as means ± SEM. $**P < 0.01$, $*P < 0.05$ vs. spontaneous delivery; $++ P < 0.01$; $+ P < 0.05$ vs. vaginal operative delivery.

for enhanced erythropoiesis after rhEPO administration [9,39–42]. During increased erythropoiesis this iron is rapidly cleared within 10–40 min, with a red blood cell uptake of 80–90% [41–45].

In our study, group III (rhEPO i.v.) showed the highest haemoglobin increase during the first 2 weeks. Surprisingly, despite the same increase of HFR and absolute reticulocyte counts we did not see the same rate of haemoglobin and haematocrit increase for group II (rhEPO s.c.). A possible reason for this observation might be different pharmacokinetics and bioavailability of i.v.- or s.c.-administered rhEPO. After i.v. administration, high erythropopietin levels are reached with a short half-life of about 6 h, while levels after s.c. administration show significantly lower peak values and a half-life of 12 h [46,47]. Bioavailability of i.v. rhEPO is considerably higher than that of s.c.-administered rhEPO and, although s.c. administration is preferred in the treatment of renal anaemia it is still questionable which is the better route of administration for a stimulation therapy with rhEPO.

While the s.c. route is preferred during substitution therapies with rhEPO, e.g. in renal anaemia, the i.v. route might be preferable for stimulation therapy where high peak EPO levels are needed to produce an additional stimulation of the bone marrow. Patient response to rhEPO stimulus shows a high individual variability and it is still not known whether the s.c. route is really more effective in stimulating erythropoiesis than the i.v. route [48–50].

The results of absolute reticulocyte count and HFR ratio indicate that both regimens stimulate erythropoiesis more than iron therapy alone.

HFR reticulocytes are the first young and immature reticulocytes to be released from the bone marrow after administration of rhEPO [28]. The increase of the HFR ratio in groups II and III reflects the additional and persistent effect of rhEPO in stimulating erythropoiesis. Hillman *et al.* [38] showed that the absolute reticulocyte count does not increase until 3–5 days and that a full level of marrow production as estimated from the absolute reticulocyte count occurs only after 8–10 days, at which time erythroid hyperplasia of the marrow and absolute reticulocyte count are increased to the same extent. By performing the reticulocyte count on days 4 and 14 we were able to detect the early release of immature reticulocytes but might have missed a difference in the absolute reticulocyte count between groups II and III around day 8.

Another reason for the major haemoglobin increase in group III could be a consequence of different ferrokinetics in this group. We could show, that under dynamic conditions, ferritin levels do not reflect iron stores but might well reflect the rate of synthesis in the reticuloendothelial system with its rapid changes under rhEPO therapy [28]. Lower increase of ferritin levels and the steep decrease of transferrin levels until day 4 might reflect the dynamic changes in the transferrin-iron pool, which are most pronounced in group III. Administration of parenteral iron causes an increase of ferritin levels and transferrin saturation within 24 h, thereafter ferritin levels usually remain elevated until 4 to 6 weeks [41,43,44,51].

As the release of iron from transferrin to the bone marrow is proportionate to transferrin saturation [33,43], the decrease of transferrin in group III might again reflect higher iron mobilization in this group.

A single dose of 100 mg of parenteral iron, which was administered to prevent functional iron deficiency, is not sufficient to prevent iron depletion during the recovery period from anaemia. Therefore additional oral iron supplementation was administered until the end of the observation period. Despite oral iron supplementation even control patients had significantly lower iron stores at day 42 – in fact, even lower than the rhEPO groups.

This shows that a single administration of rhEPO in combination with parenteral iron did not cause severe iron depletion, mean ferritin levels 6 weeks after the treatment were still in the lower normal range.

In conclusion, we were able to show that anaemic patients with low endogenous EPO levels after a postpartal haemorrhage benefit from a single administration of i.v. rhEPO. These patients might have blunted erythropoietin production owing to elevated inflammatory cytokines after delivery with or without surgical interventions. In our study, patients with low endogenous EPO levels had a significantly higher percentage of operative deliveries. Although having iron stores in the lower normal range, if they were treated with iron only, they showed a delayed increase of haemoglobin and haematocrit levels, possibly due to a lack of EPO and a block of iron transfer to the bone marrow.

By administering therapeutic amounts of rhEPO, erythropoiesis can be accelerated and in combination with parenteral iron, exogenous iron will be provided for a successful stimulation. In our study only i.v. administration of rhEPO had a significant effect in these patients. This might be due to higher EPO levels, the first day after administration and different ferrokinetics compared with the s.c. administration. However, results might be different for repeated administrations or higher dosages of i.v. or s.c. rhEPO.

There is a need for alternatives to blood transfusion in otherwise healthy patients [52]. More and more patients are refusing blood transfusion. Autologous blood donation is safe, but expensive and cannot be performed in all clinics. Also it is mostly limited to elective procedures, as in orthopaedic surgery, but not applicable for obstetrical patients, where amount and time of blood loss is not predictable. In addition, autologous blood donation might also be critical for fetal outcome. If haemoglobin increase is delayed despite iron treatment, blunted EPO production might be the underlying cause and the use of rhEPO could be taken into consideration, depending on the severity of anaemia. As iron stores are low or empty at the end of pregnancy, sufficient iron supply is mandatory. If rhEPO is used, parenteral iron administration is recommended to prevent functional iron deficiency during enhanced erythropoiesis.

References

1 Combs C, Murphy E, Laros R. Factors associated with postpartum hemorrhage with vaginal birth. Obstet Gynecol 1991;72:69–76.
2 Gilbert L, Porter W, Brown V. Postpartum haemorrhage – a continuing problem. Br J Obst Gynecol 1987;94:67–71.
3 Naef R, Chauhan S, Chevalier S, Roberts W, Meydrech E, Morrison J. Prediction of hemorrhage at Cesarean delivery. Obstet Gynecol 1994;83:923–6.
4 Gillon J, Greenburg A. Transfusion: infectious complications. Blood 1992;54:19–28.
5 Morrison J, Floyd R, Martin R, Dodson M, Roberts W, Morrison F. Blood transfusions after postpartum hemorrhage due to uterine atony. J Matern Fetal Invest 1991;1:209–12.
6 Crosby L, Palarski V, Cottington E, Cmolik B. Iron supplementation for acute blood loss anaemia after coronary bypass surgery: a randomised placebo-controlled study. Heart-Lung 1994;23:493–9.
7 Schindler E, Scholz S, Boldt J, Zickmann B, Knothe C, Dietrich G. Effectiveness of oral versus parenteral iron substitution in autologous blood donors. Infusionsther-Transfusionsmed 1994; 21:236–41.
8 Skikne B, Cook J. Effect of enhanced erythropoiesis on iron absorption. J Lab Clin Med 1992;120:746–51.
9 Suh H, Wadhawn N. Iron dextran treatment in peritoneal dialysis patients on erythropoietin. Adv Perit Dial 1992;8:464–6.
10 Erslev A. Anemia of chronic disease. In: Beutler E, Lichtman M, Coller B, Kipps T, eds. Williams Hematology. New York: McGraw Hill, 1995: 518–24.
11 Fischer C, Gill C, Forrester M, Nakamura R. Quantitation of 'acute-phase proteins' postoperatively. Am J Clin Pathol 1976;66:840–6.
12 Romem Y, Artal R. C-reactive protein in pregnancy and in the postpartum period. Am J Obstet Gynecol 1985;151:380–3.
13 Bornmann BV, Weiler J, Aulich S. Acute treatment with recombinant erythropoietin in patients with pre- and postoperative anaemia: a clinical report. Clin Invest 1994;72:S31- S35.
14 Dudrick S, O'Donnell J, Matheny R, Unkel S, Raleigh D. Stimulation of hematopoiesis as an alternative to transfusion. South Med J 1986;79:669–73.
15 Fullerton D, Campbell D, Whitman G. Use of human recombinant

erythropoietin to correct severe preoperative anemia. Ann Thorac Surg 1991;51:823–4.
16 Goodnough L, Rudnick S, Price T *et al.* Increased preoperative collection of autologous blood with recombinant human erythropoietin Therapy. N Engl J Med 1989;321:1163–8.
17 Koenig H, Levine E, Resnick D, Meyer W. Use of recombinant human erythropoietin in a Jehovah's Witness. J Clin Anesth 1993;5:244–7.
18 Koestner J, Nelson L, Morris J, Safesak K. Use of recombinant human erythropoietin (r-HuEPO) in a Jehovah's Witness refusing transfusion of blood products: case report. Trauma 1990;30:1406–8.
19 Levine E, Gould S, Rosen A, *et al.* Perioperative recombinant human erythropoietin. Surgery 1989;106:432–7.
20 Levine E, Rosen A, Gould S *et al.* Recombinant human erythropoietin and autologous blood donation. Surgery 1988;104:365–9.
21 Jelkmann W. Erythropoietin: structure, control of production, and function. Physiol Rev 1992;72:449–89.
22 Macdougall I, Cavill I, Hulme B, Bain B, Gregor EM, Kay PM. Detection of functional iron deficiency during erythropoietin treatment: a new approach. Br Med J 1992;304:225–6.
23 Macdougall I, Hutton R, Cavill I, Coles G, Williams J. Poor response to treatment of renal anaemia with erythropoietin corrected by iron given intravenously. Br Med J 1989;299:157–8.
24 Van Wyck DB. Iron management during recombinant erythropoietin therapy. Am J Kidney Dis 1989;14 (suppl 1):9–13.
25 Danko J, Huch R, Huch A. Epoetin alfa for treatment of postpartum anaemia. Lancet 1990;1:737–8.
26 Spivak J. The clinical physiology of erythropoietin. Semin Hematol 1993;30:2–11.
27 Zimmermann R, Breymann C, Huch R, Huch A. rHuEPO in the treatment of postpartum anaemia: subcutaneous versus intravenous administration. Clin Invest 1994;72:S25-S30.
28 Major A, Bauer C, Breymann C, Huch R, Huch A. rh-Erythropoietin stimulates immature reticulocyte release in man. Br J Haematol 1994;87:605–8.
29 Tanke H, Nieuwenhuis I, Koper G, Slats J, Ploem J. Flow cytometry of human reticulocytes based on RNA fluorescence. Cytometry 1980;1:313–20.
30 Tanke H, Rothbarth P, Vossen J, Koper G, Ploem J. Flow cytometry of reticulocytes applied to clinical hematology. Blood 1983; 61:1091–7.
31 Tsuda I, Tatsumi N. Maturity of reticulocytes in various hematological disorders. Eur J Haematol 1989;43:252–4.
32 Eckardt K, Kurtz A, Hirth P, Scigalla P, Wieczorek L, Bauer C. Evaluation of the stability of human erythropoietin in samples for radioimmunoassay. Klinische Wochenschrift 1988;66:241–5.
33 Huebers H, Finch C. Transferrin: physiological behavior and clinical implications. Blood 1984;64:763–7.
34 Rose E, Abels R, Nelson R, Cullough DM, Lessin L. The use of rHuEPO in the treatment of anaemia related to myelodysplasia (MDS). Br J Haematol 1995;89:831–7.
35 Faquin W, Schneider T, Goldberg M. Effect of inflammatory cytokines on hypoxia induced erythropoietin production. Blood 1992;79:1987–94.
36 Huch A, Eichhorn K, Danko J, Lauener P, Huch R. Recombinant human erythropoietin (rHuEPO) in the treatment of post partum anemia. Obstet Gynecol 1992;80:127–31.
37 Adamson J. Iron and Erythropoiesis. New York: Marcel Dekker., 1993:161–76.
38 Hillman R. Acute blood loss anaemia. In: Beutler E, Lichtman M, Coller B, Kipps T, eds. Williams Hematology. New York: McGraw Hill, 1995: 704–8.
39 Biesma D, Wiel A, Beguin Y, Kraaijenhagen R, Marx J. Erythropoietic activity and iron metabolism in autologous blood donors during recombinant human erythropoietin therapy. Eur J Clin Invest 1994;24:426–32.
40 Goodnough L, Price T, Rudnick S. Iron-restricted erythropoiesis as a limitation to autologous blood donation in the erythropoietin-stimulated bone marrow. J Lab Clin Med 1991;118:289–96.
41 Hamstra R, Block M, Schocket A. Intravenous iron dextran in clinical medicine. JAMA 1980;243:1726–31.
42 Jacobs P, Finch C. Iron for erythropoiesis. Blood 1971;37:220–30.
43 Fairbanks V, Beutler E. Iron metabolism. In: Beutler E, Lichtman M, Coller B, Kipps T, eds. Williams Hematology. New York: McGraw Hill, 1995: 369–80.
44 Henderson A, Hillman R. Characteristics of iron dextran utilization in man. Blood 1969;34:357–75.
45 Jacobs P. Clinical use of total-dose infusion of iron dextran. S Afr Med J 1985;67:837–8.
46 Kindler J, Eckardt K, Ehmer B *et al.* Single-dose pharmacokinetics of recombinant human erythropoietin in patients with various degrees of renal failure. Nephrol Dial Transplant 1989;4:345–9.
47 Mahon FM, Vargas R, Ryan M, Jain A, Perry B, Smith I. Pharmacokinetics and effects of recombinant human erythropoietin after intravenous and sunctaneous injections in healthy volunteers. Blood 1990;9:1718–22.
48 Abraham P, Peter W, Kill K, Halstenson C. Controversies in determination of epoietin (recombinant human erythropoietin) dosages. Clin Pharmacokinet 1992;22:409–15.
49 Ashai N, Paganini E, Wilson J. Intravenous versus subcutaneous dosing of epoietin: a review of the literature. Am J Kidney Dis 1993;22:23–31.
50 Barclay P, Fischer E, Harris D. Interpatient variation in response to subcutaneous versus intravenous low dose erythropoietin. Clin Nephrol 1993;40:277–80.
51 Fairbanks V, Beutler E. Iron deficiency. In: Beutler E, Lichtman M, Coller B, Kipps T, eds. Williams Hematology. New York: McGraw Hill, 1995: 490–511.
52 Morrison J, Morrison F. Rational use of blood products in obstetrics and gynecology. J Matern Fetal Invest 1994;4:147–53.